EFFECT OF AN ANABOLIC STEROID ON THE TENSILE STRENGTH OF GRANULATION TISSUE IN VARIOUS NUTRITIONAL STATES

By

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ABSTRACT

The administration of an anabolic steroid (17α-methyl-17β-hydroxy-androsta-1,4-dien-3-one) to rats improved the tensile strength of experimental granulation tissue and of healing skin wounds on the 12th postoperational day. This, however, only occurred in undernourished animals which showed a retarded development of connective tissue. This difference is also reflected in the collagen content of the granulation tissue. The treatment caused a slight increase in the tensile strength in all the feeding groups on the second postoperational day.

The anabolic steroids have gained wide use in the treatment of debilitating conditions, including postoperational states, healing of fracture and osteoporosis, because of their general invigorating effect and, specifically, because of the retention of nitrogen and calcium (for general reference, *e. g. Schwarting & Neth* 1960). The main purpose of the present experiments was to find out (1) whether the anabolic steroid treatment improves the tensile strength of the experimental granulation tissue and of healing skin wound. A suitable method for testing the tensile strength was available from other investigations (*Viljanto & Kulonen,* in press). The formation of callus in bone was studied by *Wiancko & Kowalewski* (1961), who found the tensile strength significantly increased by anabolic steroids. *Pearce et al.* (1960), however, obtained conflicting results on wound healing. Since we obtained an effect, at least, in certain conditions, the question arose (2) whether the increased strength is directly correlated with the amount of collagen synthetized and (3) whether the effect can also be demonstrated in both sexes, and in various nutritional states, which affect the formation of connective tissue (*Williamson et al.* 1951; *Pearce et al.* 1960).

MATERIALS AND METHODS

Animals and feeding groups

Altogether 210 Wistar rats (group average 82–122 g, the standard deviations in the groups in the range of 2.9–11.1 %), equal number of males and females, were divided into four feeding groups, which were subdivided as shown in Table 1 and kept in steel wire cages. The feeding was started 7 days before the operation.

Group I. The rats were allowed to eat *ad libitum*, and ate about 80–90 g/rat/day of a standard wet food. Their weight increased by 25–30 g/week. The rats were kept in pairs, *i. e.* one control and one experimental animal in each cage.

Group II. Each pair of rats was given 45 g/rat/day of wet food, which resulted in a weight gain of 10–15 g/week. Two rats, both experimental or both control animals, were kept in each cage.

Group III. The food intake was controlled more strictly than in group II. The animals received the same food 35–40 g/rat/day and increased in weight by 2–5 g/week. Two rats were kept in the same cage, one experimental and one control rat.

Group IV. In this series, groups of five animals, either experimental or control rats, were kept in one cage. Only the weight gain was recorded. During the first 8 days, the feeding corresponded to that of group III, during the 12 subsequent days to that of group II and during the last 8 days to that of group I.

The food was the standard mixture of our laboratory, prepared from corn, defatted milk solids, fat and salt mixture; once a week vegetables, cod liver oil, yeast and boiled lung were given. Before the feeding, the dry mixture was soaked with about 1½-volume of water. Drinking water was given freely. The temperature in the animal quarters was + 20° C. The animals were weighed at intervals of 3–6 days. None of the animals died during the experimental period or was obviously sick. A few implants became infected and were discarded.

Treatment with steroids

The steroid given was 17α-methyl-17β-hydroxy-androsta-1,4-dien-3-one and used as two commercial preparations, Dianabol® (Ciba AG., Basel) or Anabolin® (Medica, Helsinki). The treatment was always initiated 7 days before the operation.

Group I. The experimental animals received Dianabol® 4 mg/kg/day, subcutaneously twice a week.

Group II. Anabolin® tablets were mixed in the food to give a dosage of 1 mg/kg/day.

Group III. Similar treatment was given as in Group I.

Group IV. Two experimental subgroups (with respective control groups) were formed which received either 8 mg/kg/day of Dianabol® or of Anabolin® mixed in the food.

Operations

The granulomata were produced by means of viscose cellulose sponges (10·10·20 mm piece, dry weight 75.8 ± 6.3 mg), which were divided into two halves but sutured again in the original positions. These sponges were sterilized by boiling for 15 min and implanted subcutaneously and symmetrically under the dorsal skin of the rats, which had been anaesthetized with ether. The skin wound was sutured with cotton.

Measurement of the tensile strength and determination of collagen

The rats were killed after 2, 5 or 12 days. The tensile strength of the granulation

tissue was determined by measuring the force necessary to draw the halves apart after the sutures had been removed (*Viljanto & Kulonen*, in press). The strength of the skin wound was measured in the same way, in duplicate, from strips 1 cm broad, which had been cut off perpendicularly to the wound. Two measurements were thus obtained from the granuloma and from the skin wound in each rat.

The content of collagen was estimated from the content of hydroxyproline determined according to *Neuman & Logan* (1950).

RESULTS

The data are collected in Table 1. There is a large scatter in the tensile strengths of the skin wounds although there is a tendency to an increase in strength with treatment. In granulomata it is observed (1) that on the second postoperational day there are some groups in which the increase is statistically significant. If all granuloma data on the 2nd day are arranged as non-independent pairs, the difference between the experimental and control groups is statistically significant ($P < 0.02$). This difference levels off on the fifth day, but on the 12th day, there is a difference in the feeding group III (fasting). In the granulomata the effect of sex is slight, but in the skin wounds the effect of treatment seems better in males (the average increase of strength in treated rats is + 18 % in the males, but only + 5 % in the females).

The long-term effect thus depends on the feeding and this was obvious in group III only. From the absolute values it is evident that the formation of new connective tissue is retarded in the control animals, but that treatment with anabolic steroid tends to restore it towards the normal. The effect is also clear in the skin wounds in group III.

The data on the content of collagen of the granulation tissue shown in Table 1, confirm the view that the difference in the development of the tensile strength is due to a difference of collagen formation in the rats. It seems that in the granulomata the »ratio« of the hydroxyproline content to the tensile strength is smaller in treated animals. This occurred both in males and females and is a subject on which more investigation is necessary. Some measurements were made in our laboratory (*Leena Mikkonen*, unpublished) on the tensile strength of the collagen fibres in rat tail tendons after anabolic steroid treatment. No difference was found, but the experiments were made before the importance of restricted feeding was realized.

DISCUSSION

As mentioned above, in group III (fasting), where the effect of anabolic steroid was marked, the experimental and control rats were kept in pairs in the same cages and it is possible that the experimental rats ate more than their

Table 1.

The tensile strength of granuloma pieces and skin wounds in rats treated with anabolic steroid as described in the text. Each figure gives the average and standard deviation of a group of five rats, E treated, C control, TS-G tensile strength of granuloma, TS-W tensile strength of wound, HOPro-G hydroxyproline μg/granuloma piece, averages from 5–9 pieces of granuloma, 4–6 estimations from each. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ as tested *vs.* respective control by *t*-test.

Feeding group	Sex	C/E	Initial weight g	2nd day after operation			5th day after operation			12th day after operation			
				TS-G g	TS-W g	Weight gain g	TS-G g	TS-W g	Weight gain g	TS-G g	TS-W g	Weight gain g	HOPro-G μg
I	♂	C	99 ± 11	41 ± 11	44 ± 7	— 7	67 ± 24	119 ± 35	+25	401 ± 84	407 ± 41	+70	–
I	♂	E	98 ± 10	57 ± 5**	54 ± 14	+19	72 ± 14	140 ± 44	+32	452 ± 88	421 ± 69	+62	–
I	♀	C	122 ± 8	29 ± 9	40 ± 15	+10	63 ± 9	142 ± 28	+12	427 ± 71	385 ± 57	+20	–
I	♀	E	122 ± 9	31 ± 6	40 ± 11	+ 7	62 ± 9	131 ± 35	+24	441 ± 47	356 ± 73	+40	–
II	♂	C	105	25 ± 8	31 ± 8	+ 5	51 ± 7	65 ± 14	+12	494 ± 55	423 ± 55	+28	–
II	♂	E	106	36 ± 10*	46 ± 20	+ 6	55 ± 10	79 ± 30	+12	485 ± 40	450 ± 67	+30	–
II	♀	C	113	21 ± 7	36 ± 16	+ 6	38 ± 11	96 ± 23	+10	465 ± 81	415 ± 56	+21	–
II	♀	E	113	34 ± 12*	49 ± 12	+ 7	50 ± 21	87 ± 21	+14	464 ± 34	395 ± 48	+25	–
III	♂	C	104 ± 9	31 ± 11	51 ± 11	+ 8	42 ± 12	80 ± 26	+14	367 ± 103	275 ± 27	+18	1186 ± 41
III	♂	E	103 ± 11	38 ± 11	45 ± 7	+ 9	43 ± 14	75 ± 25	+16	543 ± 26***	363 ± 21**	+25	1310 ± 73**
III	♀	C	84	24 ± 5	40 ± 13	+ 3	41 ± 16	92 ± 14	+ 3	223 ± 71	237 ± 52	—10	648 ± 74
III	♀	E	84	32 ± 11	42 ± 9	+11	45 ± 25	113 ± 16	— 5	320 ± 70*	304 ± 37	— 7	675 ± 12*

share from the common feeding pot. The observed small difference in weight gain does not support this explanation, which is also not in agreement with the concept of increased retention of nitrogen with treatment (see *e. g. Almqvist et al.* 1961). The auxiliary experiment with group IV showed that the treatment with anabolic steroids did not augment the weight gain in rats on various diets. In this respect, both preparations were the same. We believe that the treated animals in group III received more food than their untreated pairs, but this cannot be wholly responsible for the almost normal (by comparison with group I) development of the tensile strength, since the weight gain was much less than in the normal (group I). In group II the dose of steroid was much lower than in group I and we consider that this explains the negative result.

To conclude, the positive effect of anabolic steroids on the tensile strength of granulomata and of skin wounds can be demonstrated on the 12th day in undernourished rats only, but is not directly related to the weight gain. It is not clear which part of this stimulating action is due to a direct effect on fibroblasts. The mechanism of the positive effect on the 2nd postoperational day is also obscure (treatment had at that time lasted 9 days): *i. e.* better migration of the fibroblasts into the sponge implants, the increased number of the fibroblasts in the tissues or possibly an increased amount of soluble collagen in the imbibing tissue fluid.

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